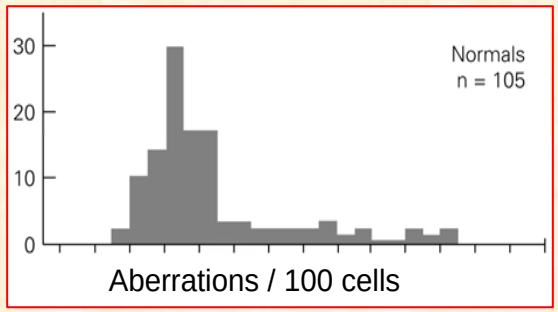
Possible High-Throughput Screening Logistics

Integrating

High-Throughput Radiation Biodosimetry

with

High-Throughput Assays for Radiation Sensitivity

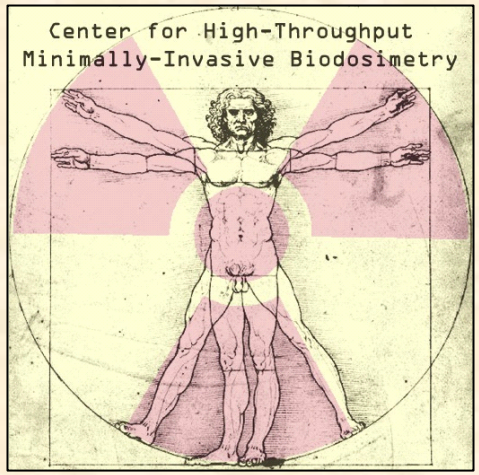


David J. Brenner

Columbia Center for High-Throughput Minimally-Invasive Radiation Biodosimetry

NIAID

Columbia Center for High-Throughput Minimally-Invasive Radiation Biodosimetry



www.cmcr.columbia.edu

Ultra high-throughput biodosimetry

In response to a radiological event, small or large, in a major US city, tens or hundreds of thousands of people will need to be screened within a few days for radiation exposure....

1) **for triage and treatment of acute radiation effects**
2) **for long term assessment of late effects (cancer, cardiac disease)**
3) **because active reassurance measures are an effective means of reducing mass panic**

Current (manual) technologies allow screening of some tens of individuals per day

Issues for an Effective High-Throughput Biodosimeter

- **Processing throughput – minimal invasiveness**
- **Sensitivity – dose coverage**
- **Specificity**
- **Processing time**
- **Signal stability**

Need for more than one approach....

Different biodosimetric endpoints needed for different situations

Our High-Throughput Radiation Biodosimetry Approaches

PROGRAM 1: Converting current biomarkers to ultra-high throughput

Micronuclei and γ-H2AX: Both already well characterized. Amenable to automation; current systems have very limited throughputs.

PROGRAM 2: Genomically-based high-throughput biodosimetry

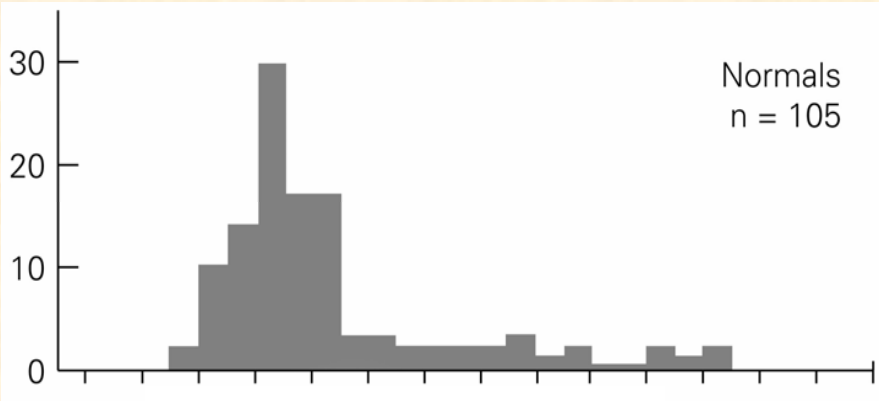
Gene expression profiling to provide a "signature" of radiation damage has been pioneered by Amundson and Fornace. The technology in a high-throughput context is new, but well advanced.

PROGRAM 3: Metabolomically-based high-throughput biodosimetry

Metabolomics (global metabolite profiles) has the potential to provide a rapid non-invasive radiation biodosimeter. The technology in a high-throughput context is well advanced.

High throughput biodosimetry is well established as critical for radiation threat countermeasures ....

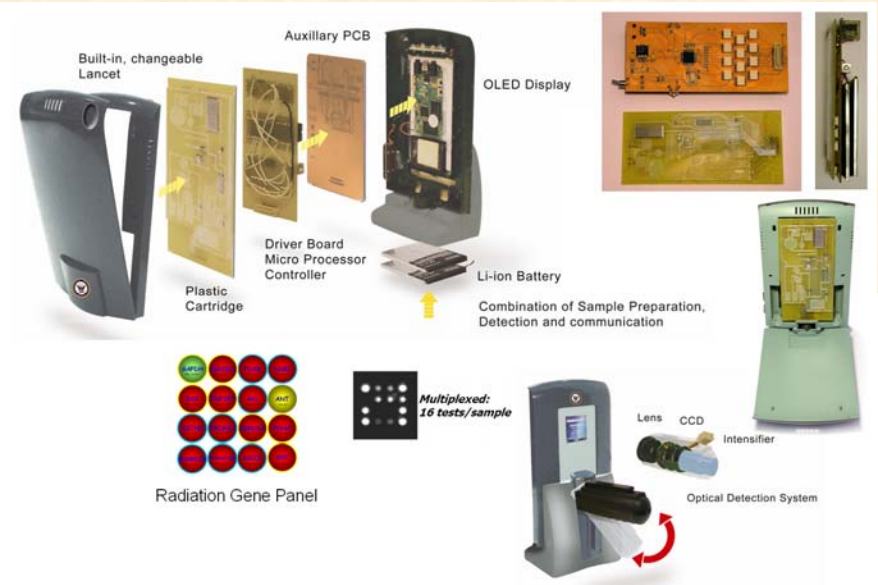
But less attention has been paid to high-throughput assays for radiation sensitivity....



....in large part because robust predictors of individual radiation sensitivity, the subject of this Workshop, have yet to be established.

In practice, how might a high-throughput assay of individual radiation sensitivity work?

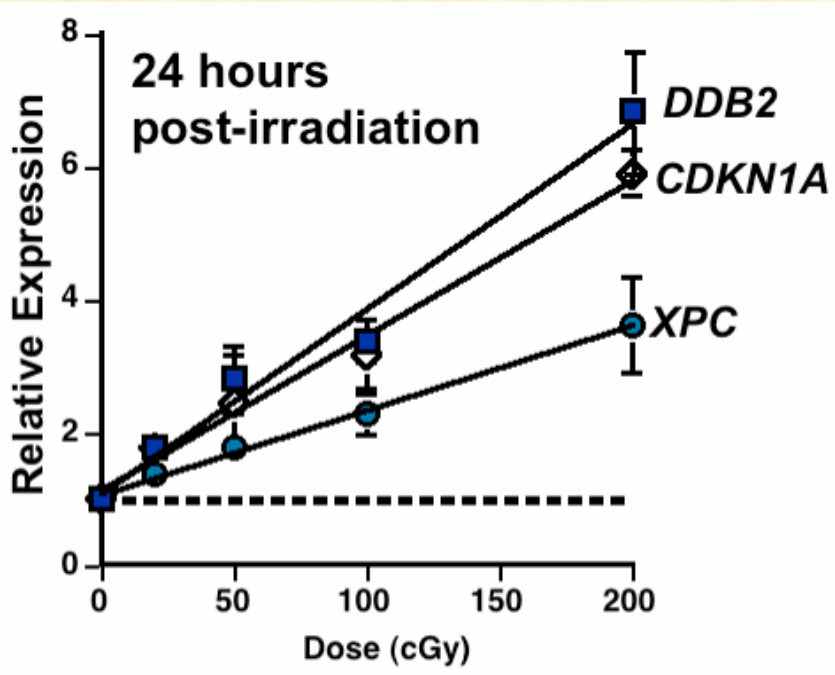
- **Could it be integrated with high-throughput biodosimetry?**



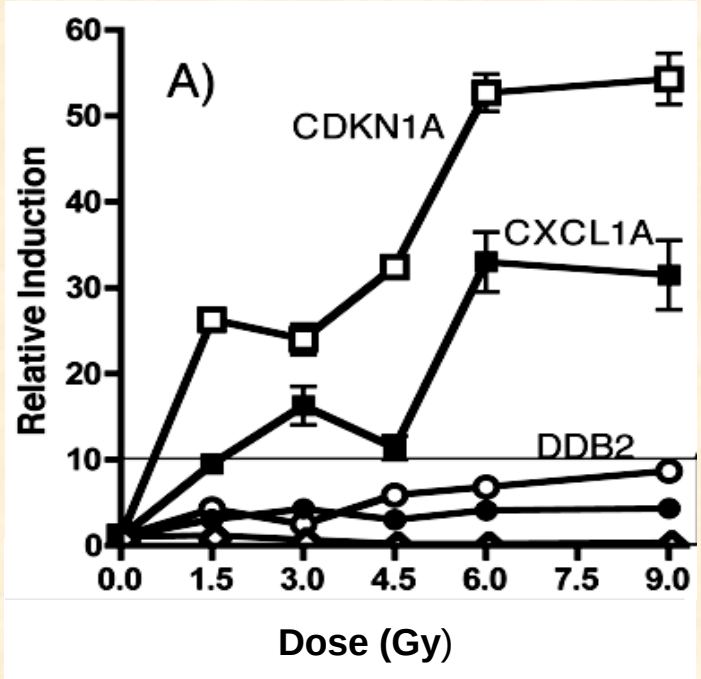
Gene expression as a basis for radiation biodosimetry

Exposure to ionizing radiation produces dose-dependent changes in the expression of many genes, potentially providing a means to assess both radiation exposure, and to quantify dose.

Ex vivo

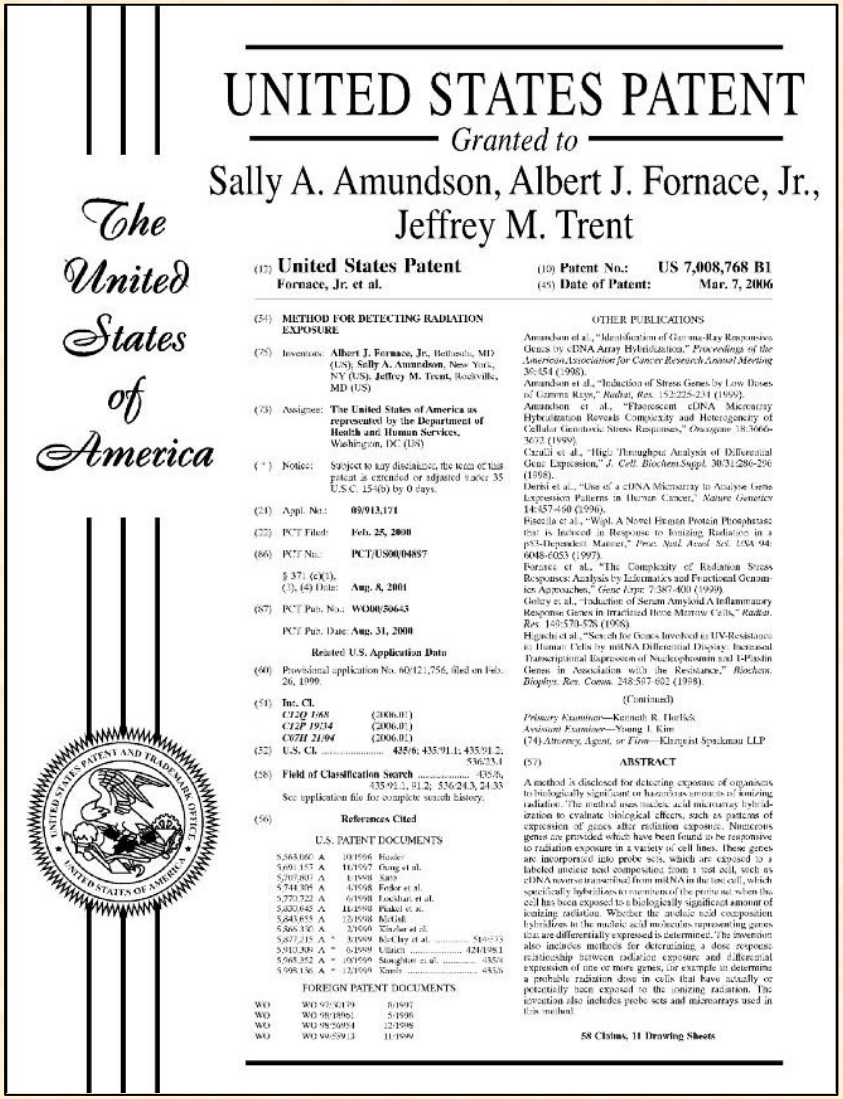


Amundson *et al*., (2000)
Radiation Research, 154 (3): 342-346



Amundson *et al*., (2004)
Cancer Research, 64: 6368-6371

We have a US patent for this approach



The United States of America

UNITED STATES PATENT

Granted to

Sally A. Amundson, Albert J. Fornace, Jr., Jeffrey M. Trent

(12) **United States Patent**
Fornace, Jr. et al.

(10) **Patent No.: US 7,008,768 B1**
(45) **Date of Patent: Mar. 7, 2006**

(54) **METHOD FOR DETECTING RADIATION EXPOSURE**

(75) Inventors: **Albert J. Fornace, Jr.**, Bethesda, MD (US); **Sally A. Amundson**, New York, NY (US); **Jeffrey M. Trent**, Rockville, MD (US)

(73) Assignee: **The United States of America as represented by the Department of Health and Human Services**, Washington, DC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/913,171**

(22) PCT Filed: **Feb. 25, 2000**

(86) PCT No.: **PCT/US00/04857**

§ 371 (c)(1),
(2), (4) Date: **Aug. 8, 2001**

(87) PCT Pub. No.: **WO00/50643**

PCT Pub. Date: **Aug. 31, 2000**

Related U.S. Application Data

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(51) **Int. Cl.**
C12Q 1/68 (2006.01)
C12P 19/34 (2006.01)
C07H 21/04 (2006.01)

(52) **U.S. Cl.** **435/6**; 435/91.1; 435/91.2; 536/23.1

(58) **Field of Classification Search** 435/6, 435/91.1, 91.2; 536/24.3, 24.33
See application file for complete search history.

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(Continued)

Primary Examiner—Kenneth R. Horlick
Assistant Examiner—Young J. Kim
(74) *Attorney, Agent, or Firm*—Klarquist Sparkman LLP

(57) **ABSTRACT**

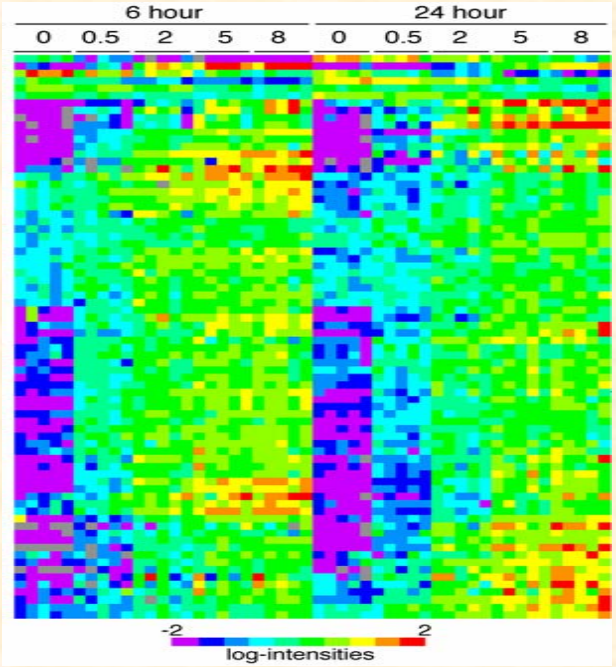
A method is disclosed for detecting exposure of organisms to biologically significant or hazardous amounts of ionizing radiation. The method uses nucleic acid microarray hybridization to evaluate biological effects, such as patterns of expression of genes after radiation exposure. Numerous genes are provided which have been found to be responsive to radiation exposure in a variety of cell lines. These genes are incorporated into probe sets, which are exposed to a labeled nucleic acid composition from a test cell, such as cDNA reverse transcribed from mRNA in the test cell, which specifically hybridizes to members of the probe set when the cell has been exposed to a biologically significant amount of ionizing radiation. Whether the nucleic acid composition hybridizes to the nucleic acid molecules representing genes that are differentially expressed is determined. The invention also includes methods for determining a dose response relationship between radiation exposure and differential expression of one or more genes, for example to determine a probable radiation dose in cells that have actually or potentially been exposed to the ionizing radiation. The invention also includes probe sets and microarrays used in this method.

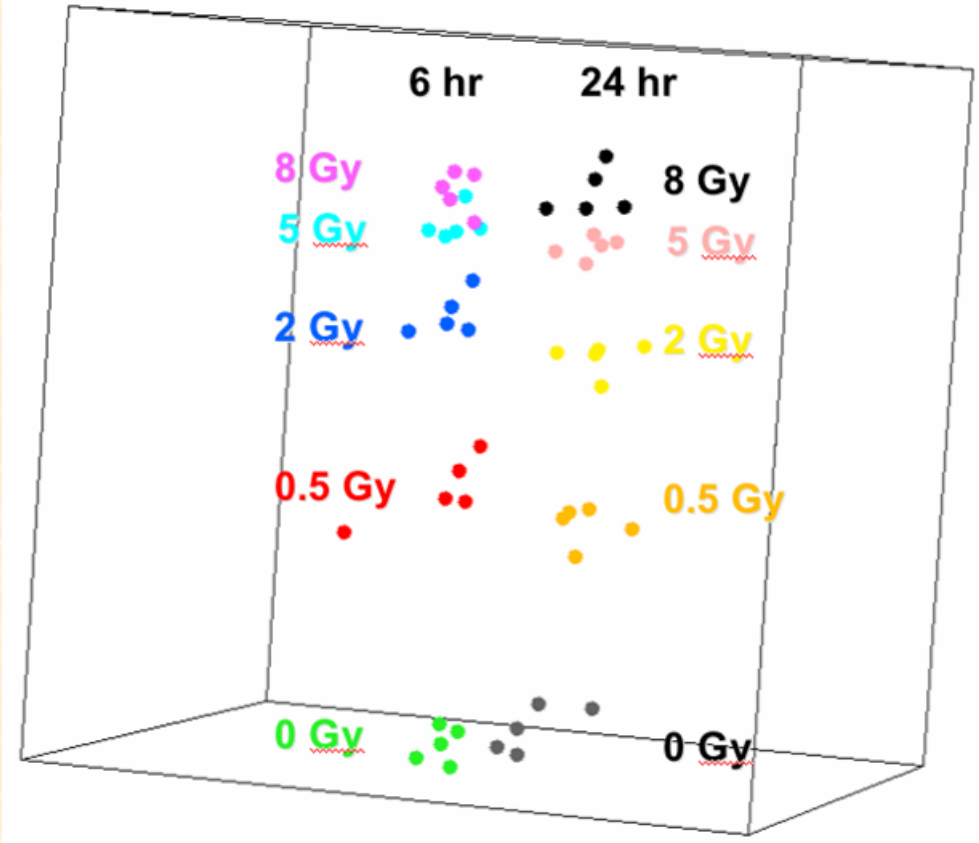
58 Claims, 11 Drawing Sheets

"METHOD FOR DETECTING RADIATION EXPOSURE"

US Patent # 7,008,768
March 7, 2006

Multi-dimensional scaling (MDS) plot of a 74-gene response profile, separating samples by dose

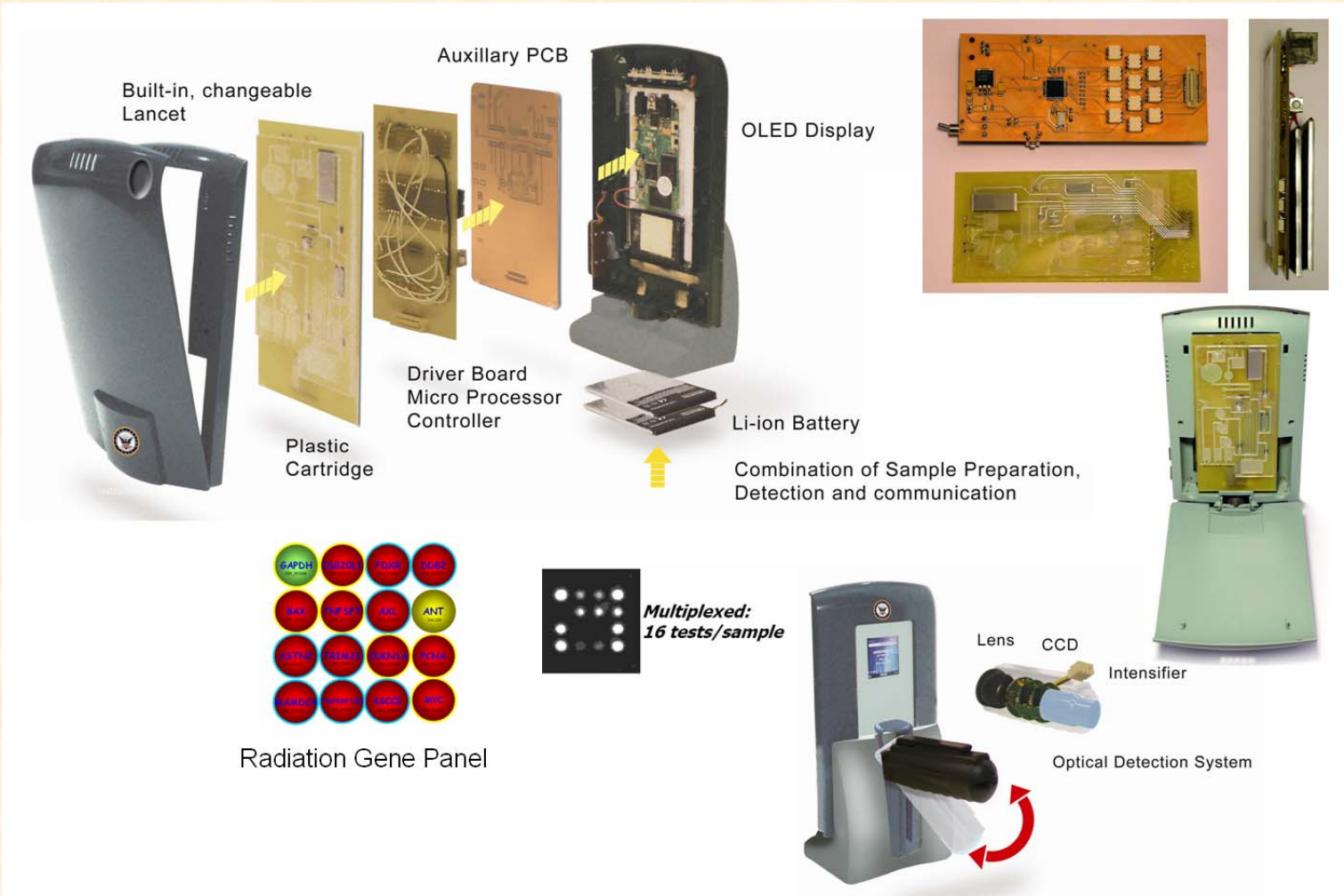




Using just a few genes does <u>not</u> give us the specificity we need

Amundson et al , 2008

Components of genomically-based high throughput biodosimetry system



Thousands of such cartridges can be stockpiled, for use after a large-scale radiological event

Quantitative Nuclease Protection Assay (qNPA) for High Throughput Genomics

Sawada et al 2006

Advantages of qNPA (quantitative Nuclease Protection Assay)

- ✓ **Whole blood is used**
  Mix with lysis buffer, run assay.

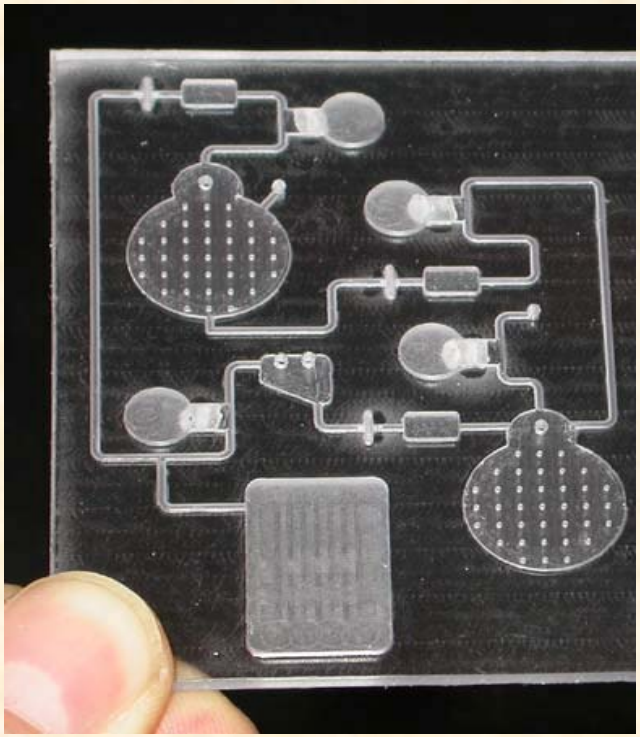
- ✓ **Directly measures gene abundance:**
  No amplifications (eg PCR) needed.

- ✓ **Multiplexed:**
  Array simultaneously measures multiple genes

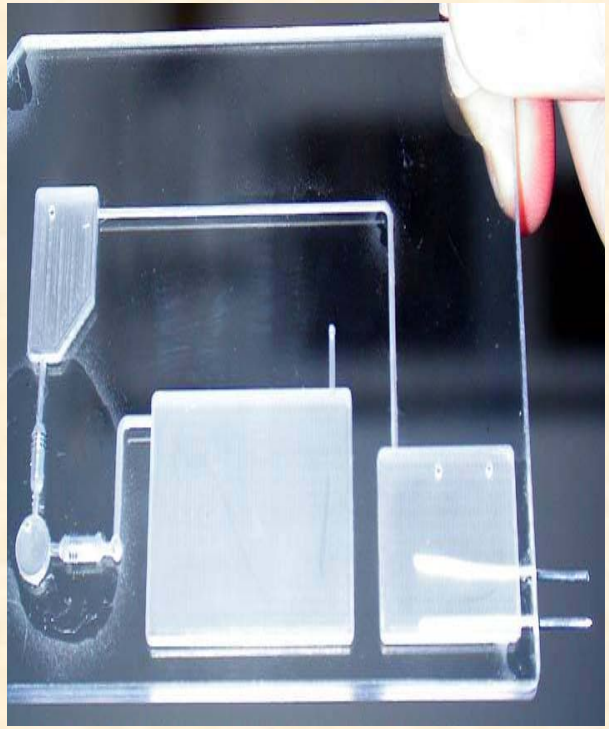
- ✓ **Reproducible and repeatable day-to-day:**
  Variability between samples ≤10%, repeatability day-to-day within 2%.

- ✓ **Programmable:**
  Change test genomic signature by use of a different set of reagents with the same microfluidic disposable array.

Sample analysis cartridge for qNPA



The *front-half* of the qNPA assay takes the place of the Nuclease Protection Plate of the standard microwell format

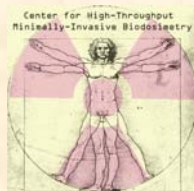


The *back half* of the qNPA assay takes the place of the Array Plate of the standard microwell format

Standalone DNA prototype

Self-contained battery operated cassette for analysis of DNA

Readily adaptable for field assessment of SNPs predicting radiosensitivity



Genomically-based identification of radiation-sensitive cancer cell lines

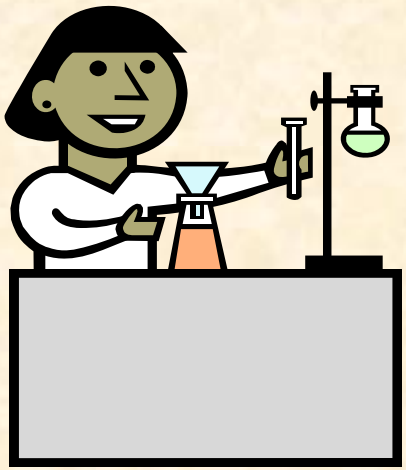
Radiation sensitive cell lines (SF2 <0.2, red points) separated by radiation-induced expression of 22 genes

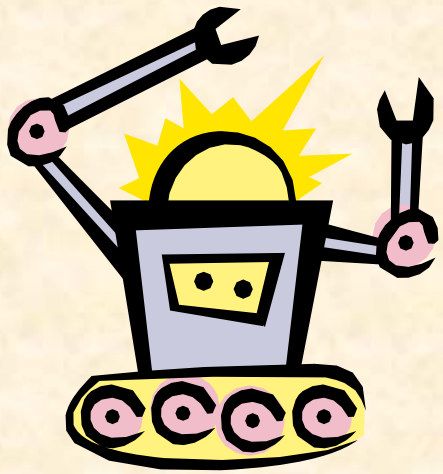
Radiation sensitive cell lines (SF2 <0.2, red points) separated by baseline expression of 175 genes

Amundson *et al*
Cancer Res. 68: 415-24 (2008)

RABIT:
Rapid Automated BIodosimetry Tool

Converting two manually-based radiation biodosimetry assays to high throughput, using a robotically-based biodosimetry workstation





RABIT:
Rapid Automated BIodosimetry Tool

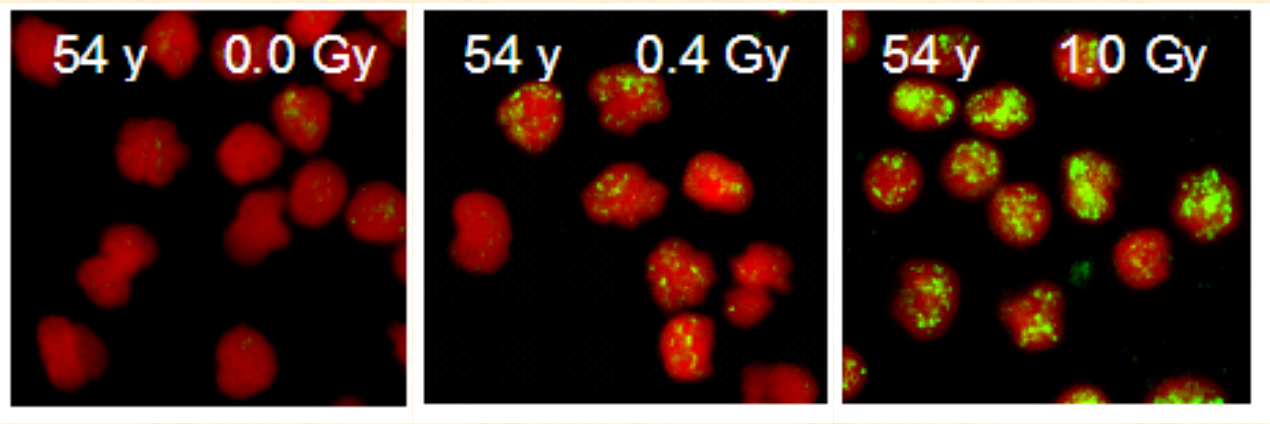
- Fully automated ultra high-speed robotic biodosimetry workstation.
- Automates two well-established manual assays, γ-H2AX and micronucleus
- One fingerstick of blood
- No human intervention

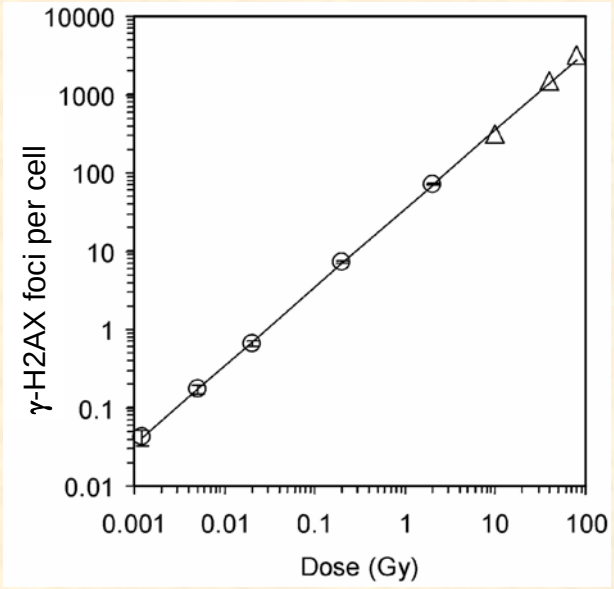
- **Phase I (2008): 6,000 samples/day**
- **Phase II (2010): 30,000 samples/day**

The main technical innovations are:

1) **Use of smaller samples – single drop of blood from a capillary finger stick**
2) **Complete automation of biology and imaging in multi-well plates**
3) **Innovations in high-speed imaging**

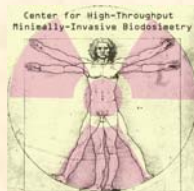
The γ-H2AX assay: A validated biomarker of radiation dose



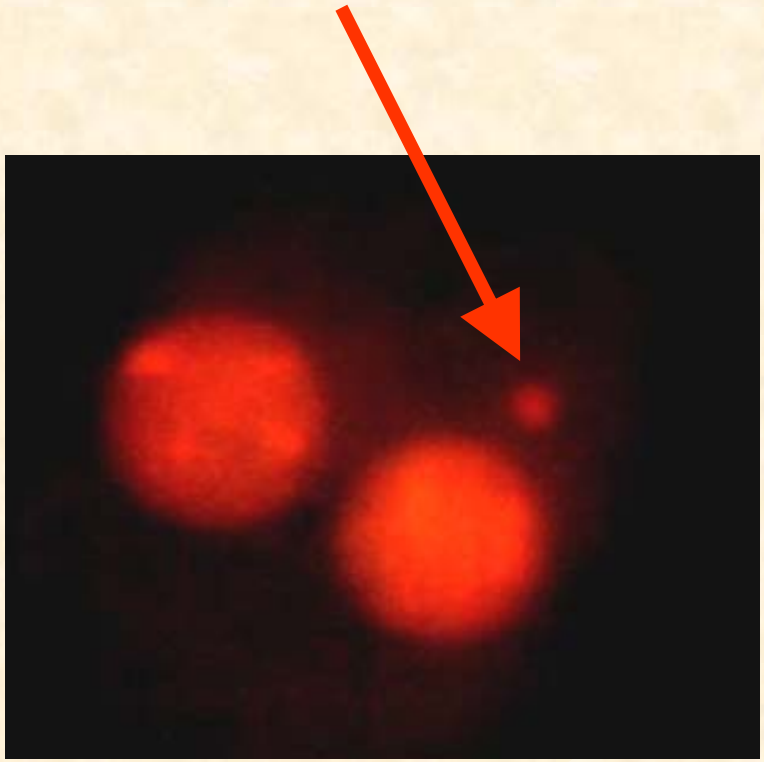


- Each green spot represents the location of a DNA repair complex
- Assay does ***not*** require culturing cells to mitosis

- Highly linear with radiation dose



Micronuclei:
A validated biomarker of radiation dose



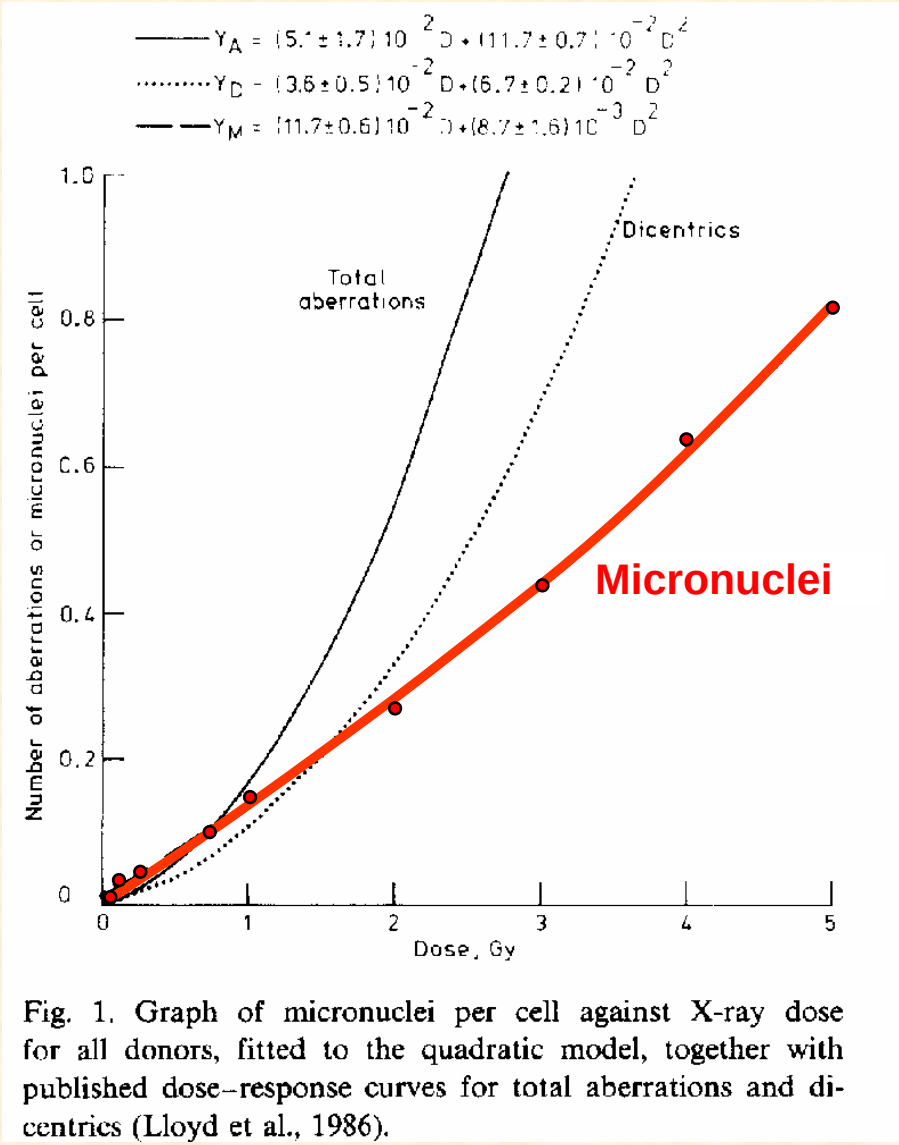
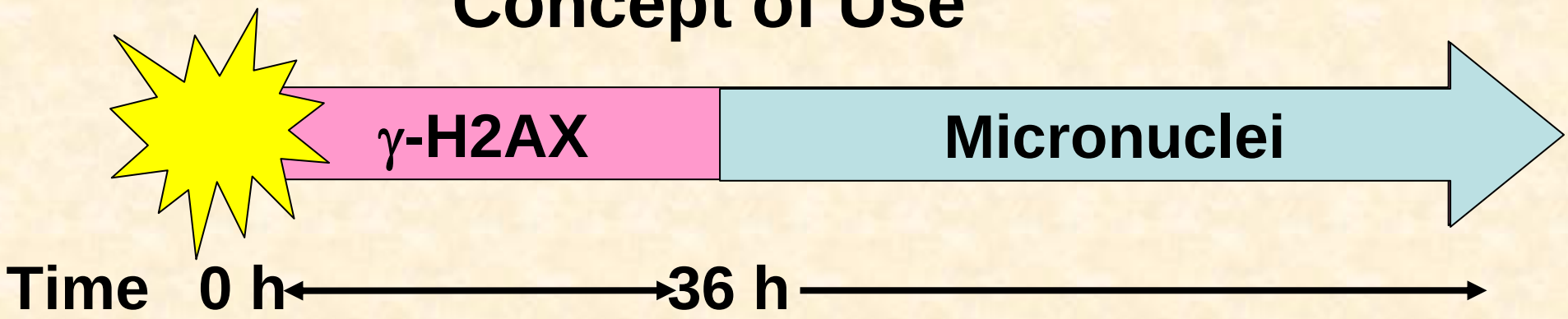


Fig. 1. Graph of micronuclei per cell against X-ray dose for all donors, fitted to the quadratic model, together with published dose–response curves for total aberrations and dicentrics (Lloyd et al., 1986).

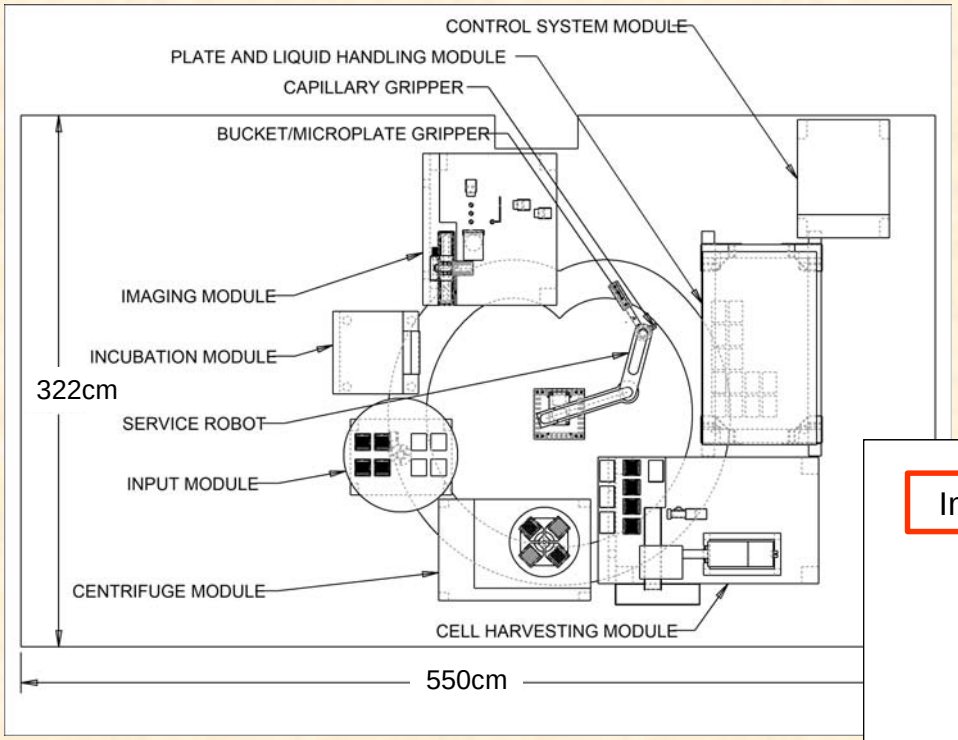
γ-H2AX vs. Micronuclei

γ-H2AX	Micronuclei
☺ Same day processing	☹ 70 hour processing
☺ Highly linear with dose	☺ Slightly non linear with dose
☹ Signal lasts only ~36 h	☺ Signal stable for years
☺ Amenable to high-throughput automation	☺ Amenable to high-throughput automation

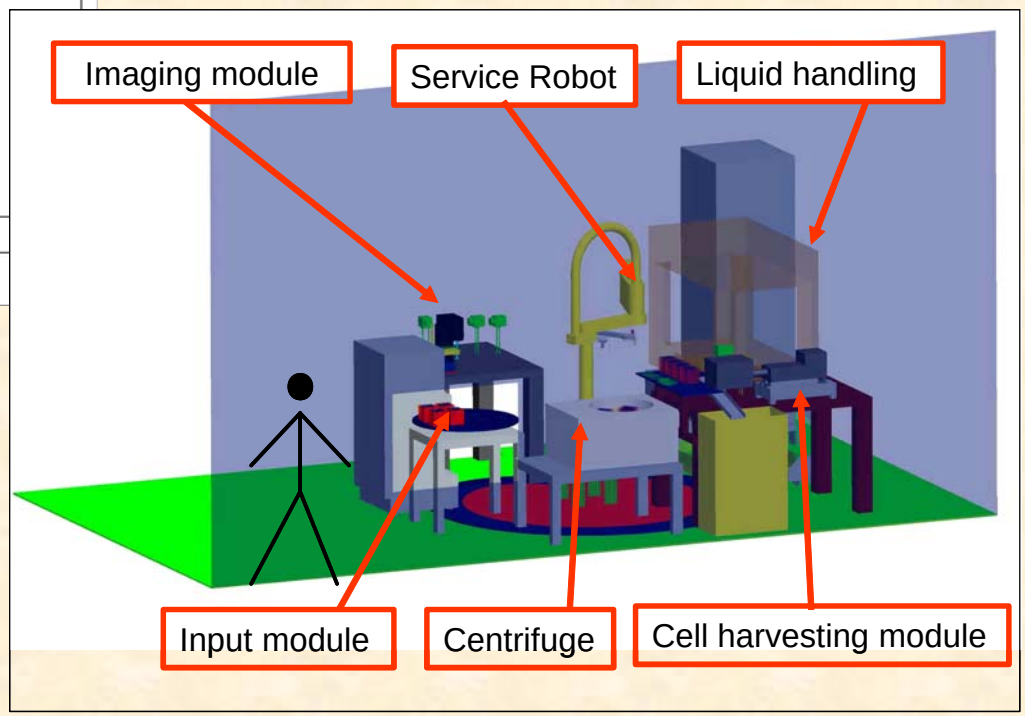
Concept of Use



RABIT device overview

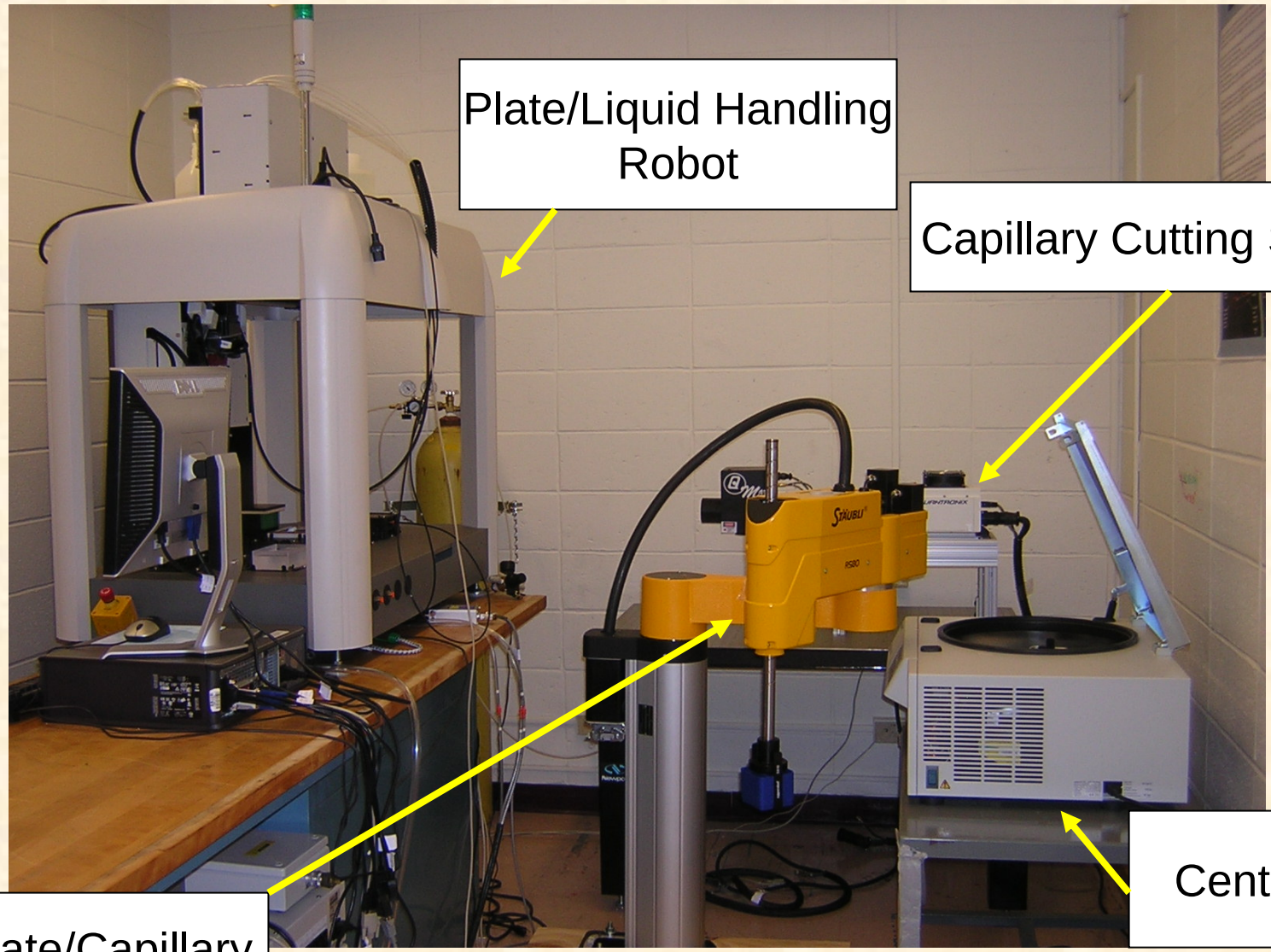


- **Modularity**
- **Truck portability (3 x 5 m footprint)**



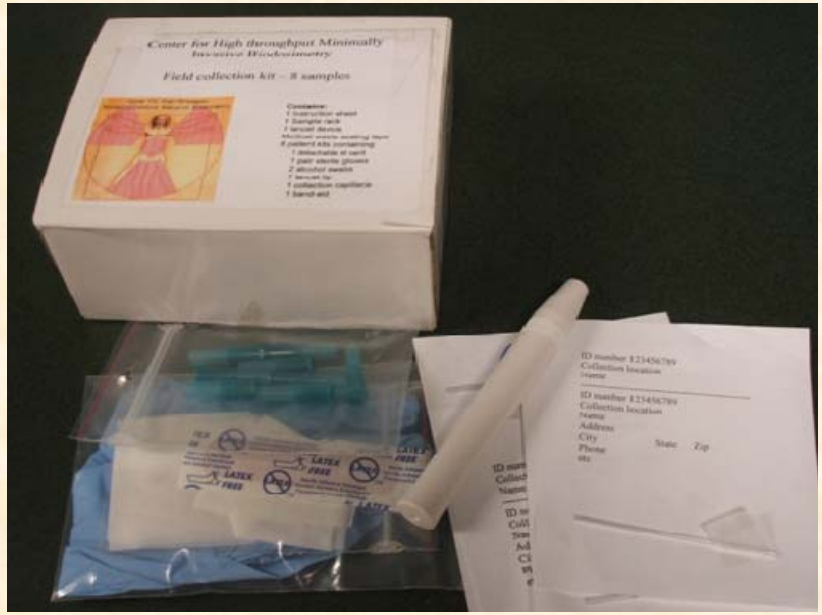
- **Full autonomy, safe human access**
- **18 hour duty cycle 30,000 samples**

Breadboard Prototype



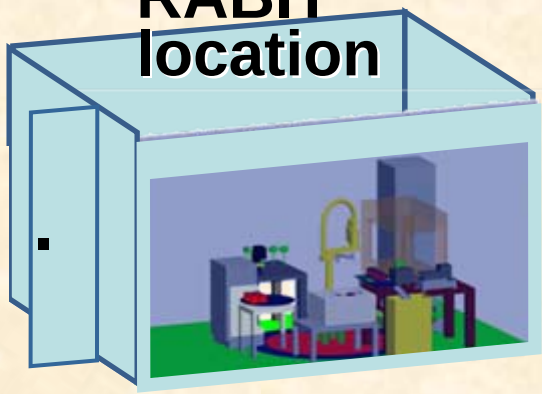
RABIT Field Collection

- We anticipate multiple collection sites, at church halls, doctor's offices, etc.
- Standard capillary lancet used to draw drop of blood (same as used for home diabetes tests)
- When filled, capillary is placed in a 24-tube holder, designed for transport to (and direct insertion in) the RABIT machine



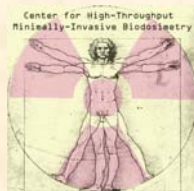
Field collection kit contains matched bar-coded capillaries, data collection cards, capillary holder, gloves, lancets, etc.

Central RABIT location

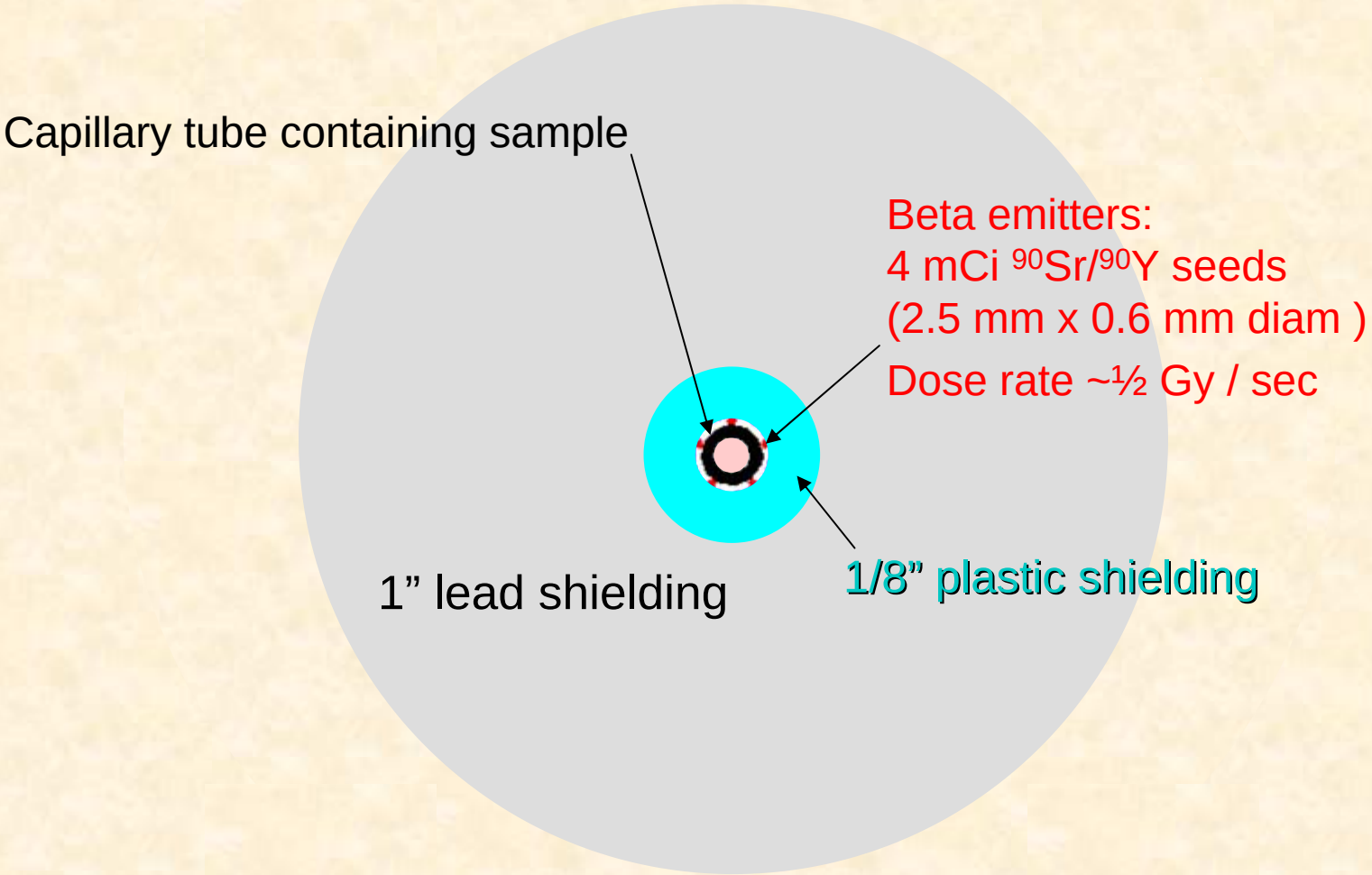


Using the RABIT to assess radiation sensitivity

- **The RABIT would not be able to assay for a mechanistically-based predictor of radiosensitivity, but a more functional approach is an option.**
- **The sample is split into two (A and B):**
  - **A is assayed for the biodosimetric endpoint of choice;**
  - **B is irradiated, in a high throughput platform, to a dose significantly higher than those anticipated from the radiological event;**
  - **Then B is assayed in exactly the same way as A.**
- **Sample B will yield a functional estimate of radiation sensitivity, to augment the dose estimate from Sample A.**

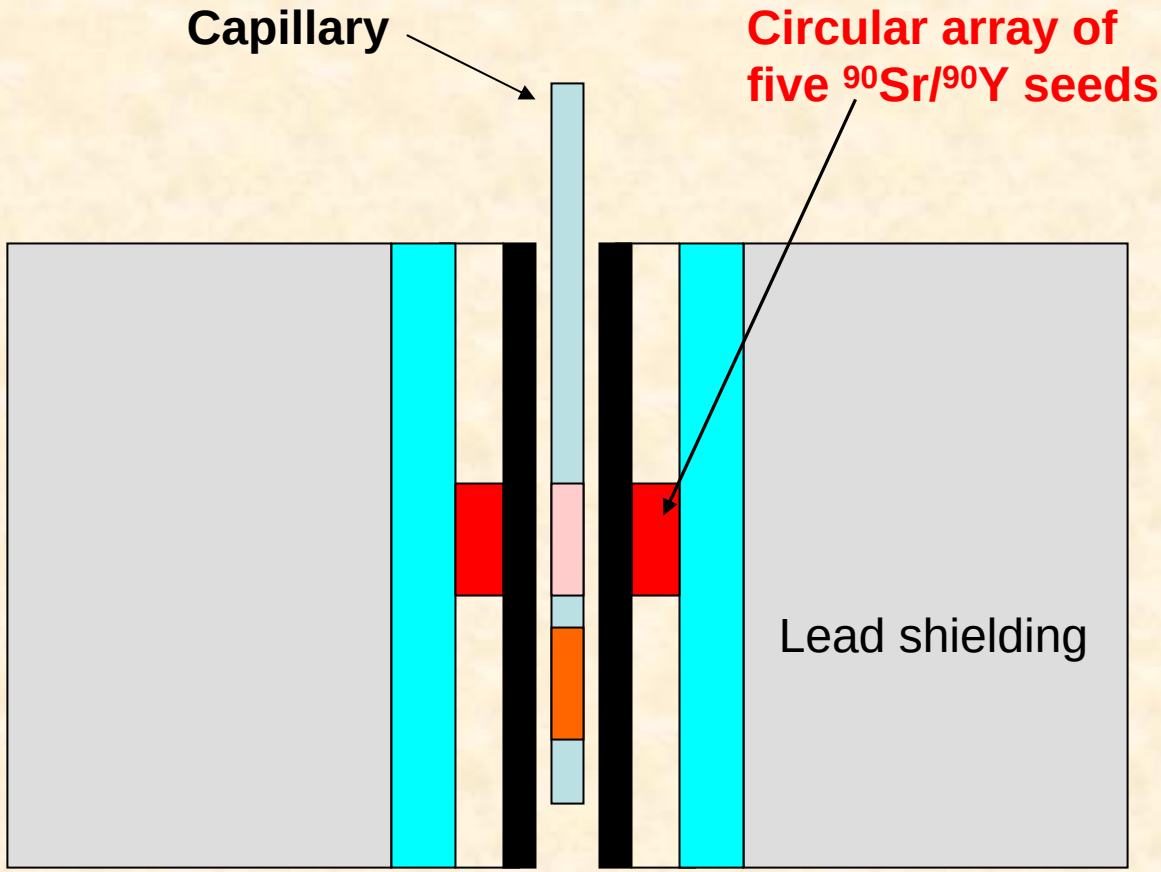


High throughput capillary irradiator



Top view

High throughput capillary irradiator



Side view, not to scale

In practice, how might a high-throughput assay of individual radiation sensitivity work?

- **Could it be integrated with high-throughput biodosimetry?**

- **Mechanistically-based genomic radiosensitivity assays could indeed be integrated**
- **Functionally-based approaches are also feasible**